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Quantification of Lipoprotein(a): Comparison of an Automated Latex-Enhanced Nephelometric Assay with an Immunoenzymometric Method

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Summary: Several studies indicate the relevance of lipoprotein(a) (Lp(a)) in the genesis of premature coronary artery disease. A simple method for determining the concentration of Lp(a) is therefore of great interest for assessing the risk of coronary artery disease in patients. We compared a new latex-enhanced immunonephelometric assay (Behringwerke AG, Marburg, Germany), using the Behring Nephelometer System 100, with an established immunoenzymometric assay (Immuno, Heidelberg, Germany). A total of 163 patients was studied. Intra- and inter-assay coefficients of variation were between 2.2% and 7.1%, and between 3.4% and 8.6%, depending on the concentration of Lp(a). The correlation between the studied assays was excellent ($r = 0.93$, $y = 0.98x - 1.57$, *Spearman* rank, *Passing & Bablok*). When values above 1000 mg/l for Lp(a) were excluded, the correlation was even higher. Increased light scattering with particle size, which hitherto has been a disadvantage of the nephelometric technique, seems to be negligible using the improved latex-enhanced approach. In patients with triacylglycerol values above 4.5 mmol/l ($n = 19$) there was no interference with the Behring system, i. e. the results of the nephelometric method were not increasing, and they agreed with those of the immunoenzymometric assay. In conclusion, this new latex-enhanced nephelometric immunoassay represents a rapid and precise method for the quantification of Lp(a).

Introduction

Several studies indicate the relevance of Lp(a) in the genesis of premature coronary artery disease (1–3). A serum concentration of Lp(a) above 300 mg/l in patients with coronary artery disease seems to be an important risk factor, especially if LDL-cholesterol is also increased (4). Lp(a) deposits are detectable in atherosclerotic lesions (5). The biochemical composition of Lp(a) is similar to that of the LDL particle, which contains apolipoprotein B-100, attached to the glycoprotein apolipoprotein(a). Apolipoprotein(a) has striking similarities to plasminogen (6–11); it exists in at least 34 different phenotypic forms (M_r 300 000 to 800 000), which are genetically determined (12–16). Lp(a) has been found to interfere with the binding of plasminogen to immobilized fibrin and to the plasminogen receptor (17). Lp(a) is therefore of major interest in studying the complex interactions between coagulation and fibrinolytic systems and dyslipoproteinaemia in relation to atherogenesis.

Several methods for the quantification of Lp(a) are commercially available for clinical diagnostic tests (18–28). However, several difficulties must be overcome in order to determine the concentration of Lp(a) specifically. Since apolipoprotein(a) has a high homology to plasminogen, it is important to use an antibody in the detection system, which has no cross-reactivity with plasminogen. The high molecular heterogeneity of Lp(a) requires polyclonal antibodies for routine use, which recognize all isoforms of Lp(a). The variable number of kringle-4 type 2 repeats in Lp(a) necessitate an antibody directed towards other than kringle-4 domains in Lp(a). Further limitations in quantitating Lp(a) are the huge size range of Lp(a) and its distribution in various density fractions within the serum. In addition, no commonly used standard is yet available for the determination of Lp(a) (30, 31).

Many of the Lp(a) kits reported so far are time- and cost-consuming. Here, we report a new simple, reliable, and fully automated method for the determination of Lp(a), using latex-enhanced immunonephelometry. The advantages and disadvantages of this system are discussed.

Materials and Methods

Fasting venous blood samples were collected for lipid analysis. Concentrations of triacylglycerols are quantitated colorimetrically by the glycerolphosphate-oxidase/peroxidase method, using a commercial standardized test kit (Boehringer Mannheim, Mannheim, Germany) (32). Serum was prepared from the blood samples. Serum samples were stored at 4 °C, and all serum samples were analysed within 24 h.

Serum was obtained from 163 patients during a period of 2 weeks. All patients came for routine lipid analysis, as inpatients or outpatients, to the Department of Gastroenterology and Hepatology of

the Medical University of Hannover. No patient was excluded from the study.

Immunoenzymometric assay

To compare and validate the nephelometric immunoassay, Lp(a) was quantitated by an immunoenzymometric assay (IEMA, Immunozym, Immuno, Heidelberg, Germany). This method is commercially available and is commonly used as a well established assay for Lp(a) (33, 34). It is a one-step immunoassay using a monovalent polyclonal antibody (from sheep) as conjugate, which is directed against the F_{ab} fragment of apolipoprotein(a), and is labelled with peroxidase. The resulting absorbance (measured at 450 nm) of the colorimetric reaction is proportional to the concentration of Lp(a). The conditions used were those recommended by the manufacturer. Lp(a) concentrations were calculated as total mass using a calibration curve obtained from a commercially available Lp(a) standard (Immuno, Heidelberg, Germany). All samples and the standards were measured in duplicate. The lower detection limit for this assay is 10 mg/l, which is defined by the manufacturer.

Latex nephelometric assay

The newly developed latex-enhanced nephelometric assay for the quantitation of Lp(a) was performed on a Behring Nephelometer 100 (BNA) (Behringwerke AG, Marburg, Germany) (22, 24, 35–40). This method is based on the latex-enhanced particle agglutination technology. The antibody is a rabbit polyclonal anti-human Lp(a) antiserum, which is conjugated to latex (N Latex-Lp(a), Behringwerke AG, Marburg, Germany). The γ-globulin fraction was isolated and purified by immunoadsorption to remove apolipoprotein B and plasminogen and subsequent ion exchange chromatography. This step is important in order to minimize the cross-reactivity of this Lp(a) assay with apolipoprotein B and plasminogen. Latex particles (polystyrol, diameter 240 nm) are conjugated with anti-human-Lp(a) γ-globulin fraction (coupling by adsorption) to enhance the turbidity of the antigen-antibody complexes. The light scattering of this complex is proportional to the concentration of the antigen Lp(a) at 840 nm. Light scattering was measured at time 0 and 6 minutes for each sample. Differences between the measured values were calculated automatically. This approach excludes non-specific light scattering. Each serum sample (30 µl) was diluted 1 : 100. The first 40 samples were also evaluated at a dilution of 1 : 400. A lyophilized Lp(a) control and standard serum were used for the quantitation (lot # SY 084101 and SY 084003, Behringwerke AG, Marburg, Germany). The reaction buffer consisted of polyethylene glycol, sodium chloride (11.6 g/l) in 0.05 mol/l phosphate buffer pH 7.0, and sodium azide (< 1 g/l). N-Diluens is a phosphate-buffered saline, pH 7.5, containing sodium azide (< 1 g/l). Samples were measured in duplicate. The lower detection limit of this assay was 30 mg/l.

Statistics

Non-parametric regression analysis was performed according to *Passing & Bablok* (41). The correlation coefficients were determined by linear regression analysis (*Spearman* rank).

Results

The patients studied were referred to us for further evaluation for their hyperlipoproteinaemia. None of the obtained sera were icteric or haemolytic as determined visually and by the qualitative detection of lipoprotein X. The calibration curve of the latex-enhanced nephelometric assay depicted in figure 1 shows a linear response. Intra-assay coefficients of variation were determined by quantitating 20 replicate samples using 8 different serum samples from patients with Lp(a) concentrations ranging from 36 to 623 mg/l using the nephelometric approach (tab. 1). The best correlation coefficients of variation (CV) were obtained within the range 75 to 471 mg/l (2.23–3.82%). This range represents the concentrations of Lp(a), which are most commonly seen in patients. In addition it is within the cutoff point (Lp(a) serum concentration of 300 mg/l) for identifying patients with increased risk for coronary artery disease. An additional sample with no detectable Lp(a) also gave a zero response in 20 runs of the nephelometric assay. The intra-assay coefficient of variation seen in the IEMA was 2.93% using pooled serum with an Lp(a) concentration of 273 mg/l (n = 20). The inter-assay variation coefficients were determined using the same serum samples on 12 consecutive days. Within the range of 71 to 455 mg/l the variation coefficients were less than 5% (tab. 1). The inter-assay coefficients of variation using the IEMA was 4.34% using pooled serum Lp(a) = 273

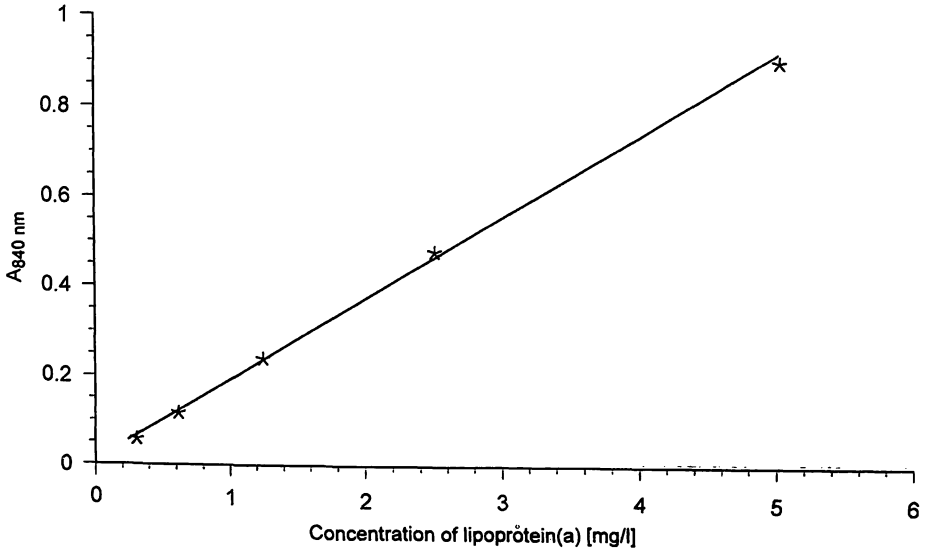

Fig. 1 The standard of 200 mg/l was diluted 1 : 40, 1 : 80, 1 : 160, 1 : 320, and 1 : 640 for the calibration curve. An internal control was determined to confirm the calibration curve. Samples were diluted to the shown range of the calibration curve to determine the concentration of Lp(a).

mg/l). Therefore, the latex-enhanced immunonephelometric method shows a good precision, which is comparable with that of the IEMA used for comparison.

Figure 2 represents the comparison of the Lp(a) quantification using the Behring system with that using the IEMA from Immuno. The mean Lp(a) concentration was 254 ± 357 mg/l (median = 90 mg/l) using the latex-enhanced nephelometric assay and 238 ± 330 mg/l (median = 106 mg/l) using the IEMA. All the studied samples revealed a highly significant correlation with r = 0.93 and y = 0.98x − 1.57. A particularly high correlation was found for values up to 1000 mg/l. To determine the influence of triacylglycerols in the latex-enhanced nephelometric assay, patients will triacylglycerols above 4.5 mmol/l (n = 19) were studied, and the results are depicted in figure 3. Although hypertriglyceridaemia is known to increase the light scattering in nephelometric assay, the latex-enhanced immunonephelometric method showed no increase in values in comparison with the studied IEMA. The mean triacylglycerol values in these patients were extremely elevated, with values of 13.4 ± 14.5 mmol/l (median = 6.3 mmol/l). Lp(a) determined in these patients were 195 ± 213 mg/l (median = 70 mg/l) using the latex-enhanced nephelometric assay versus 236 ± 286 mg/l (median = 30 mg/l) using the IEMA. These data may even reflect an underestimation of the concentration of Lp(a) by the nephelometric assay, compared with the immunoenzymometric approach in pronounced hypertriglyceridaemic patients. This might be explained by the excessive hypertriglyceridaemia observed in our studied subjects. Since we used a predilution of 1 : 100 to test this particle-enhanced method, we also compared the quantification of Lp(a) with a predilution of 1 : 400 in 50 patients. As shown in figure 4, there is a very high correlation between these two different sample predilutions (r = 0.998, y = 1.07x − 3.72).

Tab. 1 Precision of the latex-enhanced nephelometric lipoprotein(a) assay

	Lipoprotein(a) (mg/l)		CV (%)
	Mean	SD	
Intra-assay (n = 20)	35.6	2.5	7.08
	74.6	2.7	3.63
	306.6	10.6	3.44
	340.9	7.6	2.23
	419.2	15.3	3.66
	449.3	12.2	2.72
	470.5	18.0	3.82
	622.7	28.1	4.51
Inter-assay (n = 12)	37.5	3.1	8.57
	71.3	3.5	4.92
	298.3	11.0	3.69
	326.7	11.0	3.36
	402.0	15.7	3.91
	441.3	17.8	4.03
	455.3	19.7	4.32
	631.7	32.8	5.19

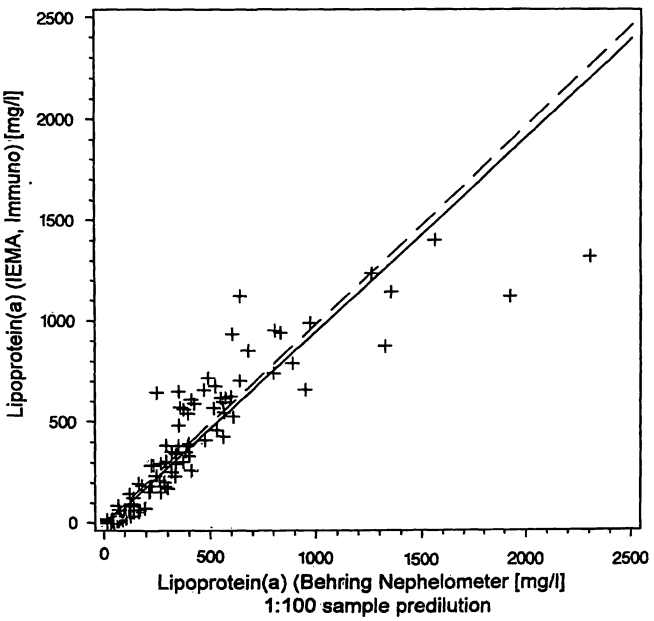

Fig. 2 Comparison of the results for the quantification of Lp(a) using the IEMA (Immuno) versus the latex-enhanced immunonephelometric technique (n = 163). The correlation coefficient is r = 0.93 and the regression line equation is y = 0.98x − 1.57, determined by *Passing & Bablok* and linear regression analysis (*Spearman* rank). In the latter 0.98 reflects the slope and 1.57 they y-intercept.

Discussion

Lp(a) shares structural similarities with LDL. It consists of one molecule apolipoprotein B and one molecule apolipoprotein(a) (42, 43). Apolipoprotein(a) is enriched with carbohydrates and displays a high degree of variable size polymorphism (13, 15, 16, 44). Apolipoprotein(a) consists of a serine-protease domain and a kringle-containing domain. The kringle domain is composed of eleven different kringle forms. One of these kringle forms has a high homology to the kringle 5 of plasminogen (6). All other kringles of apolipoprotein(a) share a high homology with kringle 4 of plasminogen (6). Kringles are structural motifs which are also found in other proteases involved in blood coagulation and fibrinolysis (6). In addition, depending on the different methods used to isolate and characterize the Lp(a) particle, there is growing evidence that the particle itself is heterogeneous (45–47). Thus, the heterogeneity of apolipoprotein(a), the differing composition of the Lp(a) particle, and the homology with other proteins and lipoproteins result in many problems for establishing a precise and reliable method for the quantitation of apolipoprotein(a) or Lp(a).

There are many reports on various methods for the measurement of the concentration of Lp(a) in human serum or plasma (48, 49). Amongst the different immunochemical approaches, immunoenzymometric assays are well

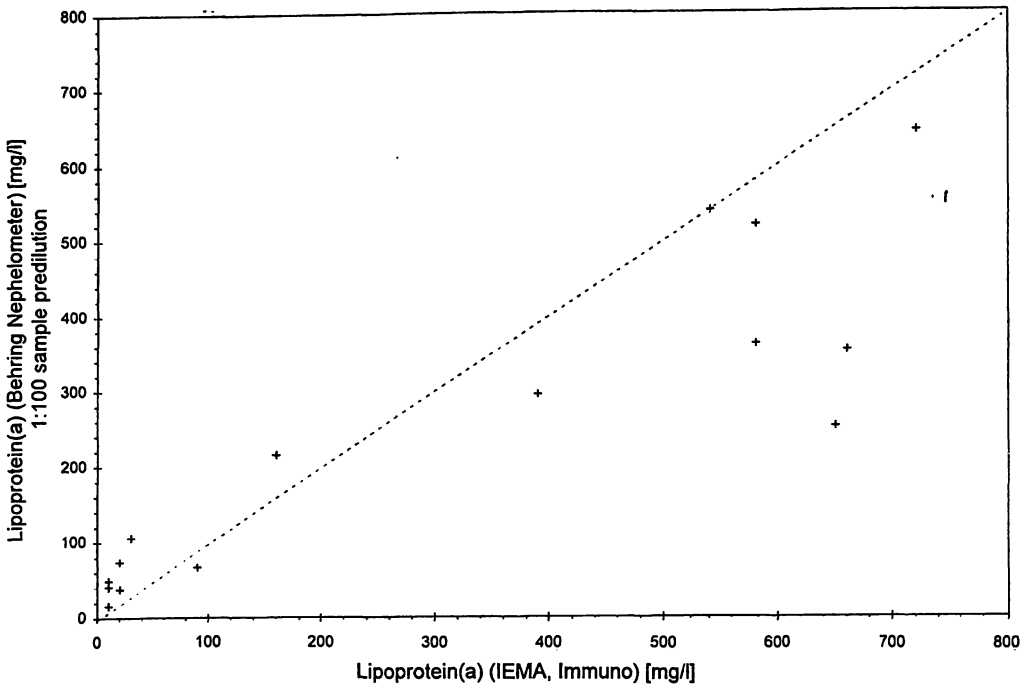

Fig. 3 The values of all patients with triacylglycerols above 4.5 mmol/l are shown. These patients had excessive hypertriglyceridaemia (13.4 ± 14.5 mmol/l (median = 6.3 mmol/l)). Lp(a) values determined in these patients were 195 ± 213 mg/l (median = 70 mg/l) using the latex-enhanced nephelometric method versus 236 ± 286 mg/l (median = 30 mg/l) using the IEMA.

established and represent the most commonly used technique for the detection of Lp(a) (26, 33, 34, 50, 51). Immunonephelometric and immunoturbidimetric assays are used increasingly, since these techniques are automatable, and therefore offer a high throughput and are cost-saving. Other advantages of the immunonephelometric and immunoturbidimetric assays are that they do not require isotopes and they are easy to perform. Recently, *Borque* et al. showed that automated turbidimetry on the Hitachi 911 analyser, using particle-enhanced technology, is improving the performance of immunoturbidimetric assays of Lp(a) (25). We used the same approach to compare the commonly used IEMA from Immuno and a latex-enhanced immunonephelometric assay on the BNA 100. The standards for Lp(a) were provided by each manufacturer. Both methods were easy to perform. Our data show high linearity, especially between 30 and 1000 mg/l, and a high correlation, with $r = 0.93$ and the regression line equation of $y = 0.98x - 1.57$. The coefficients of the intra- and inter-assay variations were very similar for both methods, with low imprecision for each. When Lp(a) values were determined in patients with excessive hypertriglyceridaemia, we found no increased values for Lp(a) using the latex nephelometric assay. Actually, in patients with increased triacylglycerol concentrations, the Lp(a) values produced by the latex-enhanced method possibly tended to be low. In addition, we assume that the wide range of Lp(a) determined in our patients also reflects the differences in Lp(a) particle size. These differences in size of apolipoprotein(a) and Lp(a) may result in different light scattering, which in turn, should result in false high values of Lp(a). Since both methods agreed well and showed high linearity (at least up to 1000 mg/l, fig. 2) for this wide range of Lp(a), we believe that the known disadvantage of increased light-scattering due to large particles when using nephelometric systems seems to be neglegible in this latex-enhanced version. However, we did not specifically set out to answer this question in the present study.

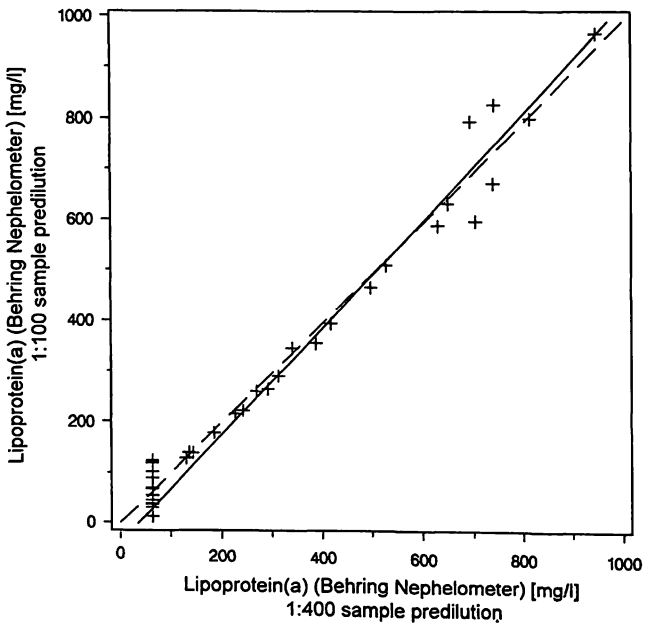

Fig. 4 Values of 50 samples determined by the latex-enhanced nephelometric method using a 1 : 100 versus a 1 : 400 predilution. The correlation coefficient is $r = 0.998$ and the regression line equation is $y = 1.07x - 3.72$).

There still remain many problems to be resolved for an international standardized precise Lp(a) or apolipoprotein(a) measurement. However, the IEMA from Immuno and the new latex-enhanced nephelometric assay from

Behring are precise, especially within the range of Lp(a) used as the cutoff to differentiate an increased risk of atherogenesis and thrombogenesis (300 mg/l). The particle-enhanced version of the immunonephelometric approach does improve the reliability of the quantification of Lp(a), and is therefore a promising method for the clinical routine. Further studies are required to establish the new generation of latex-enhanced nephelometric technology for the quantification of proteins.

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References

1. Wiklund O, Angelin B, Olofsson S-O, Erikson M, Fager G, Berglund L, et al. Apolipoprotein(a) and ischemic heart disease in familial hypercholesterolemia. Lancet 1990; 335:1360–3.
2. Armstrong VW, Cremer P, Eberle E, Manke A, Schulze F, Wieland H, et al. The association between Lp(a) concentrations and angiographically assessed coronary atherosclerosis – dependence on serum LDL-levels. Atherosclerosis 1986; 62:249–57.
3. Berg K, Dahlen G, Borresen AL. Lp(a) phenotypes, other lipoprotein parameters and familial history of heart disease in middle-aged males. Clin Genet 1979; 16:347–52.
4. Loscalzo J. A unique risk factor for atherothrombotic disease. Arteriosclerosis 1990; 10:672–9.
5. Cushing GL, Gaubatz JW, Nava ML, Burdick BJ, Bocan TMA, Guyton JR, et al. Quantitation and localization of apolipoprotein(a) and B in coronary artery bypass vein grafts resected at re-operation. Arteriosclerosis 1989; 9:593–603.
6. McLean JW, Tomlinson JE, Kuang W-J, Eaton DL, Chen EY, Fless GM, et al. cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. Nature 1987; 330:132–7.
7. Brown MS, Goldstein JL. Teaching old dogmas new tricks. Nature 1987; 330:113–4.
8. Guevara J Jr, Spurlino J, Jan AY, Yang C-Y, Tulinsky A, Venkataram Prasad BV, et al. Proposed mechanisms for binding of apo(a) kringle type 9 to apoB100 in human lipoprotein(a). Biophys J 1993; 64:686–700.
9. Philips ML, Lembertas AV, Schumaker VN. Physical properties of recombinant apolipoprotein(a) and its association with LDL to form an Lp(a)-like complex. Biochemistry 1993; 32:3722–8.
10. Huby T, Doucet C, Dieplinger H, Chapman J, Thillet J. Structural domains of apolipoprotein (a) and its interaction with lipoprotein B100 in the lipoprotein(a) particle. Biochemistry 1994; 33:3335–41.
11. Koschinsky ML, Cote GP, Gabel B, Vanderhoek YY. Identification of the cysteine residue in apolipoprotein(a) that mediates extracellular coupling with apolipoprotein B100. J Biol Chem 1993; 268:19819–25.
12. Utermann G, Menzel HJ, Kraft HG, Duba CH, Kemmler HG, Seitz C. Lp(a) glycoprotein phenotype. Inheritance and relation to Lp(a)-lipoprotein concentration in plasma. J Clin Invest 1987; 80:458–65.
13. Koschinsky ML, Beisiegel U, Henne-Bruns D, Eaton DL, Lawn RM. Apolipoprotein(a) size heterogeneity is related to variable number of repeat sequences in its mRNA. Biochemistry 1990; 29:640–4.
14. Morrisett JD, Gaubatz JW, Knapp RD, Guevara JG Jr. Structural properties of apo(a): a major apoprotein of human lipoprotein(a). In: Scanu A, editor. Lipoprotein(a). San Diego: Academic Press Inc 1990:53–74.
15. Lackner C, Cohen JC, Hobbs HH. Molecular definition of the extreme size polymorphism in apolipoprotein(a). Hum Mol Genet 1993; 2:933–40.
16. van der Hoeck YY, Wittekoek ME, Beisiegel U, Kastelein JJ, Koschinsky ML. The apolipoprotein(a) kringle IV repeats which differ from the major repeat kringle are present in variably-sized isoforms. Hum Mol Genet 1993; 2:361–6.
17. Edelberg J, Pizzo SV. Lipoprotein(a) inhibits plasminogen activation in a template-dependent manner. Blood Coagulation and Fibrinolysis 1991; 2:759–64.
18. Albers JJ, Marcovina SM, Lodge MS. The unique lipoprotein(a): properties and immunochemical measurement. Clin Chem 1990; 36:2019–26.
19. Albers JJ, Hazzard WR. Immunochemical quantification of human plasma Lp(a) lipoprotein. Lipids 1974; 9:15–26.
20. Albers JJ, Adolphson JL, Hazzard WR. Radioimmunoassay of human plasma Lp(a) lipoprotein. J Lipid Res 1977; 18:331–8.
21. Vu-Dac N, Chekkor A, Parra H, Duthilleul P, Fruchart JC. Latex immunoassay of human serum Lp(a) lipoprotein. J Lipid Res 1985; 26:267–9.
22. Cazzolato G, Prakasch G, Green S, Kostner GM. The determination of lipoprotein(a) by rate and endpoint nephelometry. Clin Chim Acta 1983; 135:203–8.
23. Levine DM, Sloan BJ, Donner JE, Lorenz JD, Henzerling R. Automated measurement of Lp(a) by immunoturbidimetric analysis. Int J Clin Lab Res 1992; 22:173–8.
24. Gillery P, Arthuis P, Cuperlier C, Circaud R. Rate nephelometric assay of serum lipoprotein(a). Clin Chem 1993; 39:503–8.
25. Borque L, Maside C, Iglesias A. Automated turbidimetry of serum lipoprotein(a). Eur J Clin Chem Clin Biochem 1993; 31:869–74.
26. Taddei-Peters WC, Butman BT, Jones GR, Venetta TM, Macomber PF, Ransom JH. Quantification of lipoprotein(a) particles containing various apolipoprotein(a) isoforms by a monoclonal anti-apo(a) capture antibody and a polyclonal anti-apolipoprotein B detection antibody sandwich enzyme immunoassay. Clin Chem 1993; 39:1382–9.
27. Kottke BA, Bren ND. A particle concentration fluorescence immunoassay for Lp(a). Chem Phys Lipids 1994; 67/68:249–56.
28. Kessler A, Schumacher M, Wood WG. Immunoluminometric assays for the quantification of apolipoprotein A-I, apolipoprotein B, apolipoprotein C-II, apolipoprotein(a) and lipoprotein(a). Eur J Clin Chem Clin Biochem 1994; 32:127–35.
29. Marcovina SM, Albers JJ, Gabel B, Koschinsky M, Gaur VP. The effect of the number of apo(a) kringle 4 domains on the immunochemical measurement of Lp(a). Clin Chem 1995; 41:246–55.
30. Albers JJ, Marcovina SM. Standardization of Lp(a) measurements. Chem Phys Lipids 1994; 67/68:257–63.
31. Labeur C, Rosseneu M, Henderson O. International Lp(a) standardization. Chem Phys Lipids 1994; 67/68:265–70.
32. Wahlefeld AW. Triglyzeride: Bestimmung und enzymatische Verseifung. In: Bergmeyer HU, editors. Methoden der enzymatischen Analyse. 3rd ed. Weinheim: Verlag Chemie 1974; 2:1878–82.
33. März W, Siekmeier R, Gross E, Gross W. Determination of lipoprotein(a): enzyme immunoassay and immunoradiometric assay compared. Clin Chim Acta 1993; 214:153–63.
34. März W, Siekmeier R, Gross W, Kostner GM. Determination for lipoprotein(a): evaluation of three methods. Eur J Clin Chem Clin Biochem 1993; 31:295–301.
35. Tiran A, Tiran B, Hojas S, Kostner GM, Wilders-Truschnig MM. Immunoquantification of lipoprotein(a): comparison of nephelometry with electroimmunodiffusion. J Clin Lab Analysis 1993; 7:256–62.
36. Riesen WF, Lenz A, Keller B, Engler H. Immunonephelometrische und immunoradiometrische Bestimmung von Lp(a). Labor-Medizin 1993; 9:326–37.

37. Borque L, Rus A, del Cura J, Maside C, Escanero J. Automated latex nephelometric immunoassay for the measurement of serum lipoprotein(a). J Clin Lab Analysis 1993; 7:105–10.
38. Fink PC, Römer M, Haeckel R. Measurement of proteins with the Behring Nephelometer: a multicentre evaluation. J Clin Chem Clin Biochem 1989; 27:261–76.
39. Buffone GJ, Lewis SA. Advantages of small angle light scattering measurement for immunonephelometry. In: Peters H, Wright PH, editors. Plasma protein pathology. Oxford: Pergamon Press, 1979:55–61.
40. Sternberg JC. A rate nephelometer for measuring specific proteins by immunoprecipitation reactions. Clin Chem 1977; 23:1456–64.
41. Passing H, Bablock W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. J Clin Chem Clin Biochem 1983; 21:709–20.
42. Fless GM, Snyder ML, Furbec JW Jr, Hedo MTG, Mora R. Subunit composition of lipoprotein(a) protein. Biochemistry 1994; 33:13492–501.
43. Sines J, Rothnagel R, van Heel M, Gaubatz JW, Morrisett JD, Chiu W. Electron cryomicroscopy and digital image processing of lipoprotein(a). Chem Phys Lipids 1994; 67/69:81–9.
44. Hixson JE, Britten ML, Manis GS, Rainwater DL. Apolipoprotein(a) glycoprotein isoforms result from size differences in apo(a) mRNA in baboons. J Biol Chem 1989; 264:6013–6.
45. Blanco-Vaca F, Gaubatz JW, Bren N, Kottke BA, Morrisett JD, Guevarà J Jr. Identification and quantification of apolipoproteins in addition to apo(a) and apoB100 in human lipoprotein(a). Chem Phys Lipids 1994; 67/68:35–42.
46. Bard JM, Delattre-Lestavel S, Clavey V, Pont P, Derudas B, Parra HJ, Fruchtart JC. Isolation and characterization of two subspecies of Lp(a), one containing apoE and one free of apoE. Biochim Biophys Acta 1992; 1127:124–30.
47. Scanu AM, Pfaffinger D, Edelstein C. Postprandial Lp(a): identification of triglyceride-rich particle containing apoE. Chem Phys Lipids 1994; 67/68:193–8.
48. Labeur C, Rosseneu M. Methods for the measurement of lipoprotein(a) in the clinical laboratory. Curr Opin Lipidol 1992; 3:372–6.
49. Marcovina SM, Levine DM, Lippi G. Lipoprotein(a): structure, measurements, and clinical significance. In: Rifai N, Warnick R, editors. Laboratory measurement of lipids, lipoproteins, and apolipoproteins. Washington DC: American Association for Clinical Chemistry Inc 1994: 235–63.
50. Usher DC, Swanson C, Rader DJ, Kremer J, Brewer HB Jr. A comparison of Lp(a) levels in fresh and frozen plasma using ELISAs with either anti-apo(a) or anti-apoB reporting antibodies. Chem Phys Lipids 1994; 67/68:243–8.
51. Fett TL, Kuhn K, Wechsler JG, Ditschuneit H. Enzyme-linked immunosorbent assay oder Elektrophorese? Ein Methodenvergleich. Labor-Medizin 1991; 14:455–9.

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